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SHORT COMMUNICATION

Expression of cytochrome P450 in yeast after different chemical treatments

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In *Saccharomyces cerevisiae* a number of chemical agents induce synthesis of cytochrome P450. A cytochrome P450 gene has been well characterized in this yeast: *CYP51*, which codes for a constitutive enzyme involved in the 14α-demethylation of lanosterol, a key step in the biosynthesis of ergosterol. In this work, we have analysed the level of transcription of the *CYP51* gene in correlation with cytochrome P450 enzymatic activity after treatment with several chemical agents known to interact with cytochrome P450. Using as a probe a DNA fragment whose identity to the *CYP51* gene was established by sequence analysis and mapping on chromosome VIII, a unique RNA species was observed in all treatment samples. The increased level found for this transcript in cells treated with ethanol, 20% glucose, phenobarbital or 5-methoxypsoralen correlates with the levels of induction in cytochrome P450 enzymatic activity measured in cells grown under the same conditions, indicating that induction of cytochrome P450 by these treatments is regulated at the transcriptional level.

Cytochrome P450 is the terminal oxidase of the monooxygenase system in all eukaryotes and bacteria (1). In higher organisms, the diversity of oxidative reactions and the broad substrate specificity are due to the presence of multiple cytochrome P450 forms coded by multigenic families (2). In *Saccharomyces cerevisiae* at least two different isoenzymatic forms have been characterized through spectral properties (3,4). The sequence of the gene coding for lanosterol 14α-demethylase (*CYP51*) has been determined (5) and, more recently, sequence analysis of a gene involved in spore wall maturation (*DIT2*) has uncovered another *CYP* gene, *CYP56* (2,6), whose expression is restricted to sporulating cells (6).

In this work we have analyzed some aspects of the induction of cytochrome P450 expression in yeast after exposure to specific chemical and physical agents such as ethanol (7,8), phenobarbital (9), 5-methoxypsoralen (10), ammonium metavanadate (11) and UV irradiation (12). These interact in different ways with cytochrome P450 and possibly induce different forms of cytochrome P450. In the present study, the levels of P450 and of ethoxycoumarin *O*-deethylase, evaluated after exposure to the different agents, were correlated with the level of induction of *CYP51* mRNA extracted from yeast cells treated with the same agents. Determination of the ethoxycoumarin *O*-deethylase activity was used to monitor the general, non-specific induction of the monooxygenase system (13).

Enzymatic levels were measured in cells *S.cerevisiae* strain D7 (*ade2-40,2-119 ilv1-92 trp5-12, 5-27*) grown in semi-anaerobic conditions. Approximately 10^9 cells were inoculated into 200 ml of complete medium containing 0.5% glucose and incubated for 3 h in the presence of 10 mM 5-methoxypsoralen, 0.2% phenobarbital, 2% ethanol or after UV irradiation (12). Alternatively, cultures were grown for 6 h in complete medium containing 20% glucose or 20% glucose + 6 mM ammonium metavanadate. For each different treatment, a sample corresponding to $7.5-12.5 \times 10^8$ cells was washed twice with water and resuspended in 3 ml of 0.8 M sorbitol, 10 mM EDTA, 50 mM Tris–HCl buffer, pH 7.4. Cytochrome P450 levels were determined directly in whole cells (14) using the reduced CO-difference spectrum measured with a Perkin Elmer λ 5 spectrophotometer. A molar extinction coefficient of 91 000 M^{-1} cm^{-1} was used. The deethylation of 7-ethoxycoumarin to umbelliferon was measured in whole cells (7) in samples corresponding to $5-10 \times 10^8$ cells, washed twice and resuspended in 15 ml of 50 mM Tris–HCl, 1.15% KCl buffer, pH 7.4. All treated cultures grown in the 0.5% glucose medium showed an increase in cytochrome P450 level and ethoxycoumarin *O*-deethylase activity with respect to the control (cells grown in 0.5% glucose). An increase was also observed in cells grown for 6 h in 20% glucose, while the addition of 6 mM ammonium metavanadate under the same conditions (6 h in 20% glucose) resulted in a decrease in the level of cytochrome P450 (Table I). Some of the chemicals used, i.e. phenobarbital and ethanol, are well-known inducers in mammals (15,16) and yeast, while UV irradiation has been shown only recently to induce cytochrome P450 in yeast (12). 5-Methoxypsoralen is also a substrate of cytochrome P450 (17), though its interaction with cytochrome P450 is still to be clarified. Ammonium metavanadate has been shown to possess a direct inhibitory effect on cytochrome P450 as it is reduced to ammonium vanadile (inside the cell) abstracting electrons to the monooxygenase system. The values of enzymatic activity and amount of cytochrome P450 shown in Table I are in good agreement with the results reported in the literature for the inhibition exerted by this compound in mammals (11).

Expression of the *CYP51* gene was analyzed by Northern blotting of total RNA extracted (18) from cells grown under the appropriate conditions for cytochrome P450 induction. Total RNA samples were separated by gel electrophoresis, transferred to a nylon membrane and hybridized to the cytochrome P450-specific DNA probe, prepared by labeling a 2.4 kb *Bgl*II fragment whose identity to the *CYP51* gene reported in the literature (5) was verified by sequence analysis. Figure 1 shows that a unique *CYP51*-specific RNA species 2100 nucleotides long is present in all samples (19). The amount of cytochrome P450-specific RNA increases after treatment with ethanol, 20% glucose, phenobarbital, 5-methoxypsoralen or UV irradiation with respect to the control grown in 0.5% glucose. The increase in mRNA observed in cells grown in 20% glucose was not significantly reduced when ammonium metavanadate was added to the culture, since this compound inhibits cytochrome P450 mainly at the protein level. It should be noted also that the UV-treated RNA sample showed an increase that does not correlate with the level of enzymatic activity observed for the

Table I. Levels of cytochrome P450 and 7-ethoxycoumarin *O*-deethylase activity in D7 strain cells after different treatments

Treatment	pmol cyt. P450/mg total protein	Sp. act. (pmol umbelliferon/mg total protein/min)
0.5% glucose (control)	ND	ND
Phenobarbital	4.7 ± 0.7	2.8 ± 0.8
5-Methoxypsoralen	12.2 ± 1.4	6.7 ± 1.1
Ethanol	12.4 ± 1.7	5.1 ± 1.6
UV irradiation	7.1 ± 1.2	3.6 ± 0.9
20% glucose	15.0 ± 1.6	7.6 ± 1.3
Ammonium metavanadate	10.9 ± 1.5	6.3 ± 0.8

For each treatment, cells were grown for 3 h in complete medium containing 0.5% glucose in the presence of 0.2% phenobarbital, 10 mM 5-methoxypsoralen, 2% ethanol or after UV irradiation. The 20% glucose and 20% glucose + 6 mM ammonium metavanadate cultures were grown for 6 h in complete medium.
Results are the mean ± SD of at least five independent experiments. ND, not detectable.

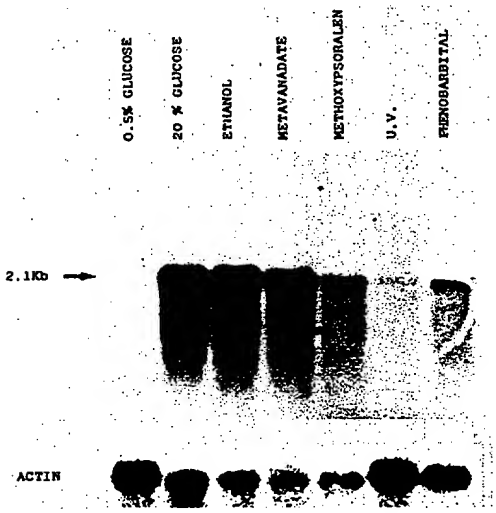

Fig. 1. Northern blot analysis of the *CYP51* transcript. Total RNA extracted from cells grown in 0.5% glucose, 20% glucose, 0.5% glucose + 2% ethanol, 6 mM ammonium metavanadate added to 20% glucose medium, 0.5% glucose + 10 mM methoxypsoralen, 0.5% glucose + 0.2% phenobarbital or from UV-irradiated cells (growth conditions were as described in the legend to Table I). RNA samples (30 μg/lane) were electrophoresed, blotted to a nylon membrane and probed with the *CYP51*-specific DNA fragment. The blot was reprobed with radiolabeled actin cDNA as a quantitative control.

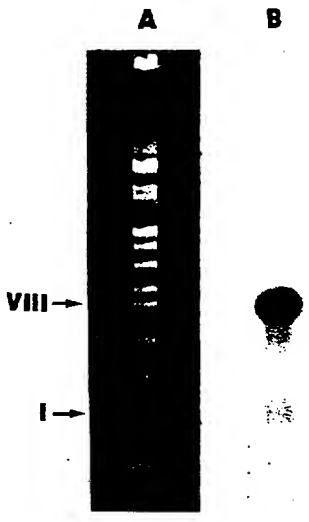

Fig. 2. Chromosome mapping of the *CYP51* probe. (A) *S.cerevisiae* chromosomes (24) were separated by transverse alternating field electrophoresis performed as recommended by the manufacturer (Beckman, Geneline) with a pulse time of 50 s for 16 h at 150 V, followed by a step with a pulse time of 120 s for 24 h at 120 V. (B) A Southern blot of chromosomes resolved by TAFE and hybridized to the *CYP51* probe.

same sample (Table I). This enzymatic activity could possibly be due to the expression of a different cytochrome P450 transcript with low homology to the probe used in this experiment but of identical size on a Northern blot. In mammals, the cDNA encoding one P450 can react with two mRNAs of identical size but transcribed from two genes of the same family. Alternatively, a low, non-specific increase of the *CYP51* transcript could have been induced by UV irradiation. It has been shown also in mammals that cytochrome P450 inducers increase the level of mRNA specifically for the corresponding enzymatic form but lower increases in mRNA levels occur when other cytochrome P450 forms are induced (20).

To confirm that the DNA fragment used as a probe for the Northern blot analysis corresponds to the *CYP51* gene previously characterized and to verify if an unlinked gene with homology to the *CYP51* probe could be detected, the same probe was hybridized to a Southern blot of *S.cerevisiae* chromosomes separated by the transverse alternating-field electrophoresis technique. As shown in Figure 2, the probe gave a strong signal with the band which corresponds to chromosome VIII. Recently, mapping to the cytochrome P450 lanosterol 14α-demethylase gene near the *SOD1* locus has been demonstrated using PCR (21). Genetic analysis of the *SOD1* gene has shown that this gene maps on chromosome VIII (22). Undoubtedly, the 14α-demethylase DNA used as a probe in this work maps on chromosome VIII. However, a weak hybridization signal corresponding to the chromosome I band could also be detected with the same probe. Since another cytochrome P450 gene, *CYP56* (6), that shows low homology to *CYP51*, has been found in the *S.cerevisiae* genome, the hybridization signal on chromosome I could represent *CYP56* or yet another cytochrome P450 gene.

In the present study, analysis of the level of transcription of the *CYP51* gene showed that the only RNA species detected was induced in all treated samples. Induction of cytochrome P450 by ethanol, 20% glucose, phenobarbital or 5-methoxypsoralen appears to be regulated at the transcriptional level. Conversely, the degree of induction for the other treatments showed discrepancies with the levels of the corresponding enzymatic activities. By analogy with the mammalian system, we speculate that expression of another cytochrome P450 gene could be more

specifically induced by the treatments which gave a lower increase of the *CYP51* transcript, or that the enzymatic activities observed after these treatments could result from post-transcriptionally regulated *CYP51* expression.

Studies on the induction of cytochrome P450 during semi-anaerobic growth have indicated that control of the levels of this enzyme was mainly transcriptional (19). Recently, characterization of the promoter region of *CYP51* has identified multiple regulatory elements. Activation or repression of transcription of this gene was shown to be regulated by oxygen and heme (23). Further investigation is now required to clarify the mode of interaction of the cytochrome P450 inducers used in this study with the transcriptional regulatory elements of *CYP51*.

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